require auditory brainstem evoked potential testing for neurological investigation in itself. If auditory brainstem evoked potential testing is done then two test intensities should be used, 30 dB for auditory screening and a high level stimulus to evaluate the morphology and latencies of the evoked potential components.

CONCLUSIONS

We recommend that all infants in special care baby units are systematically screened. From our observations of the regional register, children with a family history of deafness, congenital and perinatal infection, or malformations of the head or neck should also be screened at birth. If all such children were screened routinely at birth our findings suggest that over half the children with severe congenital sensorineural impairment could be identified at birth. In addition more children with conductive impairment who require treatment would also be detected. Of course a screening service in isolation is not sufficient. It must be linked to the full continuum of services embracing diagnostic evaluation, habilitation, family counselling, and follow up.

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Comparison of female to male and male to female transmission of HIV in 563 stable couples

European Study Group on Heterosexual Transmission of HIV

Abstract

***Objective*—To identify risk factors for heterosexual transmission of HIV and to compare the efficiency of male to female and female to male transmission.**

***Design*—Cohort study of heterosexual couples. Regular partners of HIV infected subjects were tested and both members of the couples interviewed every six months. HIV prevalence in partners was analysed according to the characteristics of the couples.**

***Setting*—Nine European countries.**

***Subjects*—563 couples comprising 156 female index patients with their 159 male partners and 400 male index patients with their 404 female partners. Partners reporting risk factors other than sexual contacts with the index patient were excluded.**

***Main outcome measures*—HIV infection in partners and high risk sexual behaviour.**

***Results*—Overall, 19 (12%) male partners and 82 (20%) female partners were infected with HIV, suggesting that male to female transmission is 1·9 (95% confidence interval 1·1 to 3·3) times more effective than female to male transmission. An advanced stage of HIV infection in the index patient (odds ratio 17·6; 4·9 to 62·7) and sexual contacts during menses (3·4; 1·0 to 11·1) increased the risk of female to male transmission and stage of infection (2·7; 1·5 to 4·9), anal sex (5·1; 2·9 to 8·9), and age of the female partner (3·9; 1·2 to 13·0 for age >45 years) increased the risk of male to female transmission. None of the 24 partners who had used condoms systematically since the first sexual contact was infected.**

***Conclusions*—Several factors which potentiate the risk of transmission through unprotected vaginal intercourse have been identified. Knowledge of these factors could be helpful for counselling patients infected with HIV and their sexual partners.**

Introduction

Several studies have examined the risk of sexual transmission of HIV from infected men to their female partners.[1-7] HIV prevalence among female partners of infected men ranges from 15% to 30% in most studies from Europe and the United States. In addition to unprotected vaginal intercourse anal sex and advanced clinical or immunological stage of HIV infection in men have been shown to significantly increase the risk of transmission.

Since many more men are infected with HIV than women in most developed countries, transmission from infected women to their male partners has been poorly studied. Even in regions where HIV is predominantly acquired through heterosexual contact few data are available. Only one large study on clients of prostitutes has been published.[8]

We present the results of a European multicentre study, the aims of which are to measure the risk of and identify the risk factors for heterosexual transmission; to compare the relative efficiency of male to female and female to male transmission; and to assess the effectiveness of counselling safer sex through the prospective follow up of couples. The preliminary results on male to female transmission of HIV have been published.[1] This paper focuses on the analysis of the risk of female

Members of the group are listed at the end of the paper.

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to male transmission, and briefly updates the results on male to female transmission.

Subjects and methods

Since March 1987 13 centres from nine European Community countries have been participating in this study. Patients infected with HIV and their heterosexual partners were recruited in hospital wards, outpatient clinics, genitourinary clinics, or local public health departments (for example, HIV screening centres, drug treatment centres). The index patient was defined as the potentially infectious person. When both members of a couple were infected, the index patient was defined as the patient with a well identified risk for HIV infection (drug user, bisexual man, transfusion recipient, previous heterosexual partner of someone known or suspected to be infected with HIV). The contact was defined as a person of the opposite sex who had had more than one sexual intercourse with the index patient within the previous year. Contacts reporting other risks of HIV infection and those with other heterosexual partners with major risks for HIV infection were excluded.

Study participants were tested and interviewed individually on entry and the contacts who were HIV seronegative were followed up every six months. At each interview the couples were counselled about the risk of HIV infection and safer sex practices. At entry to the study a questionnaire was administered by the interviewer. Questions explored the history of risk factors for HIV infection, the number of sexual partners (life time, past five years, past six months), contraceptive behaviour, use of condoms, and sexual practices before and after diagnosis of HIV infection in the index patient. If partners gave a different description of their sexual behaviour the couple was excluded. History of sexually transmitted diseases in the past five years was also obtained. Centers for Disease Control classification of clinical status[9] and lymphocyte counts for index cases were obtained from medical records. We defined an index case with an advanced state of HIV infection as a case presenting clinical symptoms (fitting stage IV of the Centers for Disease Control classification) or no symptoms but a T4 cell count below 200×10^6/l.

Presence of antibodies in HIV was determined by enzyme linked immunosorbent assay (ELISA) and confirmed by western blotting or radioimmunoprecipitation in the laboratories of the participating centres. The most recent available serological result was used for the analysis. When seroconversion occurred in a contact after enrolment, the couple's behaviour in the period between the last negative and the first positive test result was analysed. Two contacts with the same index case were considered as belonging to two independent couples.

Univariate analysis was performed for categorical variables with the Fisher's exact test and χ^2 test and for continuous variables with Student's t test. Multivariate analyses were computed by logistic regression and allowed calculation of adjusted odds ratios with their corresponding 95% confidence intervals. The duration of the relationship and factors found to be associated with an increased risk of transmission in the univariate analysis were included in the model.

TABLE I—*HIV antibody status of 151 male partners and 388 female partners according to characteristics of couples*

	% (No) HIV positive	
	Male partners (n=151)	Female partners (n=388)
Mode of infection of index case:		
Intravenous drug use	13 (13/99)	18 (46/250)
Heterosexual contact	9 (3/35)	27 (11/41)
Transfusion recipient	33 (3/9)	23 (3/13)
Bisexual contact		32 (21/65)
Haemophilic patient		10 (1/10)
Unknown	0 (0/8)	0 (0/9)
Contraceptive behaviour:		
No regular contraceptive	12 (10/86)	20 (43/212)
Oral contraceptive	18 (7/40)	23 (26/114)
Intrauterine device	10 (1/10)	28 (7/25)
Condom*	0 (0/11)	18 (6/33)
Unknown	25 (1/4)	0 (0/4)
Duration of relationship (months):		
≤12	9 (4/45)	25 (16/63)
13-48	9 (6/68)	20 (31/154)
>48	24 (9/38)†	20 (34/168)
Unknown		33 (1/3)
Stage of HIV infection (CDC classification and T4 counts):		
II or III, T4>200×10⁶/l	3 (3/89)	20 (39/199)
II or III, T4 counts unknown	4 (1/25)	6 (5/86)
II or III, T4≤200×10⁶/l	40 (2/5)	50 (7/14)
IV	42 (13/31)‡	32 (25/78)‡
Unknown	0 (0/1)	55 (6/11)
Unprotected sexual contacts during menses:		
Never	8 (8/95)	20 (39/196)
At least once	20 (11/55)‡	23 (43/191)
Unknown	0 (0/1)	0 (0/1)
Unprotected anal sex:		
Never	12 (12/102)	14 (40/295)
At least once	15 (7/48)	46 (42/92)‡
Unknown	0 (0/1)	0 (0/1)
Unprotected oral sex:		
Fellatio:		
Never	12 (3/26)	11 (9/82)
At least once	13 (16/125)	24 (73/306)§
Cunnilingus:		
Never	10 (4/39)	17 (13/77)
At least once	14 (15/111)	22 (67/301)
Unknown	0 (0/1)	20 (2/10)
Painful vaginal contacts:		
Never	12 (16/134)	20 (66/322)
Occasionally	25 (2/8)	20 (10/49)
At least 50% contacts		46 (6/13)‖
Unknown	11 (1/9)	0 (0/4)

Eight male and 16 female partners who reported systematic use of condoms since the first sexual contact are not included.
CDC=Centers for Disease Control.
*Used for more than 50% of vaginal contacts.
†$p<0.02$, >48 versus ≤48 months (Fisher's test).
‡$p<0.05$ in univariate and multivariate analysis (see text for exact p values).
§$p<0.01$ (Fisher's test).
‖$p<0.04$ often versus never or occasionally (Fisher's test).

Results

By March 1991 a total of 563 couples had been enrolled. There were 156 female index cases with 159 male contacts and 400 male index cases with 404 female contacts. At recruitment 16 male and 75 female contacts were found to be HIV positive. In addition, seroconversions occurred in three male and seven female contacts after enrolment in the study.

Most of the index cases were injecting drug users or former drug users. The other transmission groups were less represented in the study sample (table I). The mean age was 27·9 (range 16 to 58) years for women and 31·3 (range 18 to 64) years for men. The median duration of the relationship for the couples was three years, ranging from two weeks to over 20 years, and the median frequency of sexual contact was three times a week.

Assuming that no risk factors for transmission would be relevant during consistent condom use, eight male and 16 female contacts who were still negative and had systematically used condoms since the first sexual contact with the index case were excluded from the analysis of risk factors. The analysis was thus undertaken on 151 male and 388 female contacts.

FEMALE TO MALE TRANSMISSION

All the male contacts who were HIV positive had had unprotected vaginal intercourse with the index women. In the univariate analysis (table I) none of the following was found to be associated with an increased risk of transmission: mode of acquisition of HIV for the woman, age of woman and contacts, frequency of sexual contact, oral sex, anal sex, use of chemical or physical contraceptives (other than condoms).

The clinical status and T4 counts of the index case were found to be strongly linked with the risk of

TABLE II — *Univariate and multivariate estimates of risk for factors associated with HIV antibody status in 151 male partners*

	No of partners		Odds ratio (95% confidence interval)	
	HIV positive	HIV negative	Univariate	Multivariate*
Stage of HIV infection of index case:				
II or III (T4>200×10⁶/l or unknown)	4	110	1·0	1·0
IV or T4≤200×10⁶/l	15	21	19·6 (5·4 to 78·7)	17·6 (4·9 to 62·7)
Unknown	0	1		
Sexual contacts during menses:				
Never	8	87	1·0	1·0
At least once	11	44	2·7 (0·9 to 8·1)	3·4 (1·0 to 11·1)
Unknown	0	1		

Eight male partners reporting systematic use of condoms since the first sexual contact are not included.
*The other variable included in the model was duration of relationship.

TABLE III — *Univariate and multivariate estimates of risk for factors associated with HIV antibody status in 388 female partners*

	No of partners		Odds ratio (95% confidence interval)	
	HIV positive	HIV negative	Univariate	Multivariate*
Stage of HIV infection of index case:				
II or III (T4>200×10⁶/l or unknown)	44	241	1·0	1·0
IV or T4≤200×10⁶/l	32	60	2·9 (1·6 to 5·2)	2·7 (1·5 to 4·9)
Unknown	6	5		
Anal sex:				
Never	40	255	1·0	1·0
At least once	42	50	5·4 (3·0 to 9·4)	5·1 (2·9 to 8·9)
Unknown	0	1		
Age of female partner				
≤45 years	75	294	1·0	1·0
>45 years	7	9	3·0 (1·0 to 9·3)	3·9 (1·2 to 13·0)
Unknown	0	3		

Sixteen female partners reporting systematic use of condoms since the first sexual contact are not included.
*Other variables included in the model were duration of relationship, painful vaginal contacts, practice of oral sex.

transmission. The prevalence of infection among contacts of women with an advanced stage of HIV infection was 42% (15/36) compared with 3% (3/89) among contacts of asymptomatic women with T4 counts over 200×10⁶/l and 4% (1/25) among contacts of asymptomatic women with unknown T4 counts (table I).

Infected contacts had had longer relationships than non-infected contacts (mean 50 months (infected) *v* 33 months (uninfected), p=0·01). Sexual contacts during menses was the only sexual practice found to be associated with an increased risk of transmission (p=0·04).

We investigated history of genital infections in the past five years. Seventy couples reported the occurrence of at least one of the following genital infections: herpes, syphilis, other ulcers, gonorrhoea, chlamydia, candidiasis, trichomoniasis. History of candidiasis in the index woman was the only genital infection associated with HIV infection in the contact; among 10 index women reporting a history of candidiasis, four had infected their partners compared with seven out of 83 women in couples with no history of genital infection (p=0·01). It is important to note that candidiasis was reported in the "other infections" item of the questionnaire and thus not systematically investigated.

Histories of candidiasis were not included in the model for multivariate analysis because such episodes were not systematically reported. The two factors found to be independently associated with HIV seropositivity of the male contact were sexual contacts during menses (odds ratio 3·4; 95% confidence interval 1·0 to 11·1; p<0·03) and an advanced stage of HIV infection in the index woman (17·6; 4·9 to 62·7; p<0·0001) (table II). Women in an advanced stage of HIV infection tended to have had longer relationships (mean 46 months advanced *v* 32 months (non-advanced); p=0·02). When the stage of the disease and the duration of relationship were included in the model the association between duration of relationship (treated either as a categorical or continuous variable) and HIV transmission disappeared.

The prevalence of HIV among male contacts was 1% (1/74) for couples presenting neither of the identified risk factors, 16% (10/63) for those presenting one of the risk factors, and 57% (8/14) for those presenting both.

MALE TO FEMALE TRANSMISSION

Three factors were found to be significantly associated with an increased risk of transmission in the univariate and multivariate analyses (tables I and III). Index men in an advanced stage of HIV infection seemed to be more infectious (adjusted odds ratio 2·7 (1·5 to 4·9); 0·001). The practice of anal sex showed a 5·1-fold (2·9 to 8·9; p<0·0001) increase in risk. Female contacts over 45 years of age were infected significantly more often than younger contacts (44% (7/16) aged >45 *v* 20% (75/369) aged ≤45; adjusted odds ratio 3·9; 1·2 to 13·0; p<0·03).

In the univariate analysis a significant association was found between oral sex and HIV transmission (p<0·01), but this association disappeared after adjustment for anal sex, probably because of a strong association between oral and anal sex. Pain during vaginal contacts was also found to be associated in the univariate analysis (p<0·04) but not in the multivariate analysis. Given the small number of women reporting pain during sex, this could be due to the lack of power of the tests. Furthermore, among five women who did not report any of the identified risk factors but who had had their first lifetime sexual contact (defloration) with the index case less than 12 months before the interview, two (40%) were found to be positive for HIV.

Among 218 couples who did not report any symptoms or diagnosis of genital infection, 16% (35) of the female contacts were infected. Rates of HIV infection significantly higher than 16% were found when a history of candidiasis was reported by the female contact (42% (10/24), p=0·005), a history of chlamydia (or non-gonoccocal urethritis for the man) was reported by either or both partners (56% (5/9), p=0·01), and a history of syphilis by both partners (75% (3/4), p=0·02). Histories of gonorrhoea, genital warts, or genital herpes reported by one or both members of the couples were not found to be associated with risk of transmission.

Estimations of transmission rates according to the level of risk were 10% (23/226) for couples presenting none of the three risk factors identified in the multivariate analysis, 31% (40/127) for couples presenting one factor, and 54% (19/35) for couples presenting at least two of the factors.

EFFICIENCY OF MALE TO FEMALE AND FEMALE TO MALE TRANSMISSION

Eighty two of the 404 female contacts were found to be infected with HIV, representing a crude transmission rate of 20% (16% to 24%). Compared with a crude rate of female to male transmission of 12% (19/159; 7% to 17%), male to female transmission was twice as efficient (odds ratio 1·9 (1·1 to 3·3)).

A significant interaction was found between the clinical and immunological status of the index case and the direction of transmission; although the rate of transmission was not different from men or women in advanced stages of HIV infection, transmission from asymptomatic men was 5·0 (1·7 to 14·7) times more efficient than from asymptomatic women.

Discussion

We observed a rate of female to male transmission of 12% and a rate of male to female transmission of 20%. Since specific characteristics such as the proportion of index cases in late stages of the disease and the

proportion of couples engaging in high risk sexual practices may vary considerably according to the study sample, crude rates observed in other studies may differ.

It is unlikely that we misclassified male to female transmission as female to male transmission as all index women in the 19 couples in whom transmission occurred presented well documented risk factors for HIV infection whereas their male contacts denied any risk other than heterosexual contact. Since most of the index cases were drug users or former drug users one possible bias could be the enrolment of contacts who had been infected through injecting drug use without reporting it. However, the proportion of infected contacts was similar for couples in whom the index case was and was not a drug user, suggesting that inclusion of unrecognised drug users was rare. Thus any effect resulting from this potential bias would be minor.

Male to female transmission seemed to be twice as effective as female to male transmission. This agrees with results obtained for other sexually transmitted diseases such as gonorrhoea, for which male to female transmission seems two to three times more effective than female to male transmission.[10]

RISK FACTORS

Although unprotected vaginal intercourse was the common risk factor for transmission in all couples we identified several factors which further increase this risk. The increase in infectivity of people positive for HIV in an advanced stage of the disease has been suggested by several authors.[1 4 6] Since the clinical state of the index case remains a risk factor regardless of the duration of the relationship the length of the relationship (and therefore the duration of exposure to the risk of transmission) is unlikely to be a confounding factor. Increased quantities of viral particles in genital secretions of patients in late stages of HIV infection (D Anderson *et al*, and Y Henin *et al*, sixth international conference on AIDS, San Francisco, 1990) support these results. Given the low number of infected male contacts, the influence of the stage of the disease on female to male transmission cannot be estimated precisely but it seems to be very strong—significantly stronger than for male to female transmission.

Anal sex has been shown to increase the risk of male to female transmission in our and other studies.[1 3] Although the role of sexual contacts during menses has been suggested (N S Hellmann *et al*, sixth international conference on AIDS, San Francisco, 1990), sample sizes in published studies on female to male transmission have not allowed examination of high risk sexual practices. Because HIV is more easily recovered from blood cells than from genital secretions a higher quantity of viral particles may be present in the vagina of HIV positive women during menses.[11] It should be noted, however, that these high risk sexual practices (anal sex and sex during menses) were not essential for transmission. Indeed, 40 out of 82 infected women never practised anal sex and eight out of 19 infected men never had intercourse during their partners' menses.

The role of previous genital lesions is difficult to interpret. Because the questionnaire recorded history of genital infections during the past five years and the median duration of the relationships was only three years some of the reported infections might have occurred before the beginning of the relationship. Thus negative results should be interpreted with caution. We found a significant association with a history of candidiasis in female index cases or contacts, and with histories of chlamydia or non-gonococcal urethritis and syphilis in couples with a male index case. We cannot exclude better reporting of genital infections in couples in whom transmission occurred due to a recall or interviewer bias, or both, especially for non-specific lesions such as candidiasis or non-gonococcal urethritis. Because all sexually transmitted diseases, including HIV infection, have the same mode of transmission, and because the prevalence of genital infections might be increased in people infected with HIV due to immunodeficiency, it is difficult to design studies to examine the independent effects of other genital infections as cofactors for HIV transmission.[12] Nevertheless, several studies in Africa and in developed countries have presented strong arguments to support the hypothesis that genital infections, especially ulcerative infections, are cofactors for transmission.[12-14]

The hypothesis that an increased fragility of the genital mucosa in perimenopausal women increases their risk of infection is reinforced by the higher risk of infection found for female contacts aged over 45 years. Similarly, the possible role of defloration suggested in this study and in previous case reports[5 15] adds weight to the argument that all factors (traumatic, infectious, hormonal) able to impair the genital mucosa might be considered as factors which increase the risk of sexual transmission.

PREVENTION

Couples using condoms as their main method of contraception may use them in association with the rhythm method rather than systematically. Among these 44 couples, six female contacts were found to be positive for HIV, suggesting that non-systematic use of condoms has a poor protective effect against HIV transmission. On the other hand, among the 24 couples systematically using condoms for HIV prevention, no contacts were found to be HIV positive.

Besides the basic risk of transmission through vaginal contact we have identified several factors which may increase the risk of transmission. Other factors that affect infectivity and susceptibility remain to be determined. Virological and immunological parameters may account for differences in infectiousness and susceptibility and should be studied to provide a better understanding of transmission mechanisms.

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Is *Bordetella pertussis* clonal?

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Abstract

***Objective*—To establish whether *Bordetella pertussis* is essentially clonal.**

***Design*—Analysis of restriction fragments of *XbaI* digests of DNA from clinical and control isolates of *B pertussis* by pulse field gel electrophoresis.**

***Materials*—105 isolates of *B pertussis*: 67 clinical isolates from throughout the United Kingdom and 23 from Germany (collected during the previous 18 months); vaccine strains 2991 and 3700; and 13 control isolates from Manchester University's culture collection.**

***Main outcome measures*—Frequency of DNA types according to country of origin and classical serotyping.**

***Results*—17 DNA types were identified on the basis of the variation in 11 fragments, banding at 200-412 kilobases; 15 types were found in the clinical and control isolates from the United Kingdom and seven in those from Germany. There was no correlation with serotype. DNA type 1 was the commonest overall (22/105 strains, 22%), predominating in serotypes 1,2 and 1,2,3 and including the vaccine strains but not the isolates from Germany.**

***Conclusions*—Current infections due to *B pertussis* are not caused by a clonal pathogen as multiple strains are circulating in a given population at one time. There is also considerable epidemiological variation in the pathogen population between countries. These findings may have implications for the design of acellular vaccines.**

Introduction

Previous work based on examining isolates of *Bordetella pertussis* by analysing allelic variation in structural genes encoding 15 enzymes by electrophoresis concluded that this pathogen was clonal and that its genetic diversity was limited.[1] This is important in vaccine development as *B pertussis* is still a major pathogen, causing over 600 000 deaths annually, one every 52 seconds.[2,3] Most deaths occur in unimmunised infants, and in the developing world only a third of children have been immunised. Control of the disease is mainly by immunisation, a process which is dependent on having a safe, effective vaccine. The original whole cell vaccine has been criticised for its associated persistent neurological effects,[2] and in the United Kingdom this has led to a fall in immunisation and the resurgence of epidemics in 1978-9 and in 1982.[4,5]

These problems have been partially offset by the development of acellular vaccines, which have been introduced in Japan as both primary and booster doses.[6] The incidence of adverse reactions was reduced, but estimates of the efficacy of these vaccines varied widely and were as low as 69%[7] compared with that for the whole cell vaccine of 80-95%.[8]

The success of an acellular vaccine is linked with the idea that isolates are sufficiently similar that part of one isolate can produce adequate immunity against infection due to the clinically important strains of that species. If *B pertussis* is essentially clonal then this should be achievable.

This concept disagrees with the finding that *B pertussis* has three separate serotypes, designated 1,2; 1,2,3; and 1,3 according to the combination of agglutinogens demonstrated.[9] Whole cell vaccines lacking agglutinogen 3 did not protect against infection due to strains of serotype 1,3.[10]

We describe DNA fingerprinting of isolates of *B pertussis* by pulsed field gel electrophoresis, in which large DNA fragments are separated by alternatively switching the current between two sets of electrodes set at an obtuse angle.[11,12] Simple electrophoresis of restriction fragments of whole DNA produced by the enzymes *Eco* RI, *Sma* I, *Nci* I, *Bam* HI, *Ava* I, or *Bgl* II had failed to discriminate between isolates (unpublished observations). The DNA was digested with *Xba* I as the genome of *B pertussis* has a G+C content of 67·7-68·0 mol%[13] and the recognition sequence of this enzyme contains the rare tetranucleotide CTAG.[14] Thus the enzyme was likely to produce DNA fragments of the appropriate size. The technique was applied to 105 isolates of *B pertussis* to determine whether the pathogen is clonal and whether variation in genotype correlates with variation in serotype. Isolates representing strains currently causing infection in the United Kingdom and Germany were examined and the results compared with 13 control isolates.

Materials and methods

Isolates—The following isolates were examined: 67 isolates from 14 public health laboratories and 16 district general hospitals throughout the United Kingdom, including four pairs of isolates from family clusters; vaccine strains 2991 and 3700; 13 isolates from this university's collection of bacteria (controls); and 23 isolates from Professor Enders, Stuttgart, Germany. The isolates were identified on the basis of

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